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By
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This is to certify that this thesis titled “Clinical profile of Philadelphia negative myeloproliferative neoplasms in India” is the bonafide work of the candidate Dr. Sachin Suresh Jadhav during the period from August 2008 to August 2011 as a part of fulfillment towards the Degree of Doctor of Medicine (higher specialty) in Clinical Hematology towards the examinations to be conducted by the Dr. M.G.R. Medical University in August 2011.

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CLINICAL PROFILE OF PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS IN INDIA

ABSTRACT

Abstract

Background and objectives: Philadelphia negative chronic myeloproliferative neoplasms (MPN) consist of polycythemia vera (PV), essential thrombocytosis (ET) and primary myelofibrosis (PMF). Clinical information, from India, on these diseases is limited. We sought to undertake a descriptive study of these disorders with emphasis on the thrombotic complications. *Methods:* A retrospective chart review of patients who had presented to our institution and had undergone testing for JAK2 mutation analysis was undertaken. *Results:* A total of 227 patients were analyzed and the most common diagnosis was polycythemia vera, 42%, followed by PMF 21% and ET 13.7%, and 12% of patients had probable MPN. Overall there was a male predominance (78.9%), except in patients with ET. Thrombosis (19.4%) and splenomegaly (11.7%) were the commonest presenting features. Arterial thrombosis was present in 35.77% of patients with probable MPN, 29.9% of PV and 25.8% of ET. Venous thrombosis was present in 17.8% of patients and was most frequent in ET (22.6%). The prevalence of JAK2 positivity was PV 66.7%, ET 58.1% and PMF 47.9%. Hydroxyurea and venesection were the commonest first-line treatment modalities. Most of the patients had at least a partial response to treatment. *Conclusions:* Polycythemia vera was the commonest MPN and the incidence of thrombosis was high in the group studied. JAK2 positivity in PV was lower than what has been reported.

INTRODUCTION

Introduction

The common Philadelphia negative myeloproliferative neoplasms (MPNs) are polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).[54] These present with clinical manifestations and consequent complications of 'myeloproliferation' such as a high hemoglobin, WBC count, platelet count, splenomegaly and thrombosis, respectively. The JAK2V617F mutation is a recurrent, clonal abnormality seen with varying prevalence in these disorders it leads to constitutive tyrosine phosphorylation activity that promotes cytokine hypersensitivity.[8] Indian data on these diseases is limited[82-89] and hence we sought to undertake an analysis of the patients who had presented to us for evaluation of these diseases.

REVIEW OF LITERATURE

Review of Literature

The common Philadelphia negative myeloproliferative neoplasms (MPNs) are: [54]

1. Primary myelofibrosis (PMF)
2. Essential thrombocythemia (ET)
3. Polycythemia vera (PV)

These disorders present with 'myeloproliferation' such that either the myeloid, red cell, or the platelet series, respectively, are proliferating. Although the pathogenesis of these disorders is based on mutations, e.g. the JAK2 mutation, which lead to the proliferation of these elements in the bone marrow, the clinical manifestations of these mutations are varied. The cause of this 'phenotypic variability' is not completely understood.

Current classification and terminology of MPNs, as per the World Health Organization (WHO) classification 2008

The current WHO classification (2008) of tumours of hematopoietic and lymphoid tissues made the following changes in the terminology and classification of the myeloproliferative neoplasms.

1. The term "disease" was replaced by "neoplasm" and hence "Chronic myeloproliferative disease [CMPD]" was replaced by "myeloproliferative neoplasm [MPN]" "Myelodysplastic syndrome/myeloproliferative disease [MDS/MPD]" was replaced by "Myelodysplastic syndrome/myeloproliferative neoplasm [MDS/MPN]".
2. Mast cell disease (MCD) was formally included in the "MPN" category.
3. Chronic eosinophilic leukemia-not otherwise specified [CEL-NOS] has been maintained within the MPN category.

4. The fact that the phenotypic diversity of MPNs is due to differences in the specific genetic rearrangements or mutation(s) that underlie the clonal myeloproliferation has been recognized and hence "Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, and *FGFR1*" has been included as a separate category which includes molecularly distinct subcategories. This is especially so since those patients who harbour a *PDGFR* mutation, respond to Imatinib. On similar lines, the diagnostic criteria for PV, ET, and PMF incorporate molecular markers such as *JAK2* and *MPL* mutations.

   The details of current diagnostic criteria of PV, ET and PMF have been mentioned in appendix on page 83.

Polycythemia vera[79]

Polycythemia vera is a chronic myeloproliferative neoplasm characterized by increased red blood cell production independent of the mechanisms that normally regulate erythropoiesis. The reported annual incidence of PV increases with advanced age and varies from 0.7 to 2.6 per 100,000 inhabitants in Europe and North America, but it is much lower in Japan. There is a slight male preponderance with the M: F ratio ranging from 1-2:1. The median age of diagnosis is 60 years, and patients younger than 20 years old are only rarely reported. The underlying cause is unknown in most cases. A genetic predisposition has been reported in some families. Ionizing radiation and occupational exposure to toxins have been suggested as possible causes in occasional patients.

Three phases of PV may be recognized:

(1) A prodromal, pre-polycythemic phase characterized by borderline to only mild erythrocytosis

(2) An overt polycythemic phase, associated with a significantly increased red cell mass, and

(3) A "spent" or post-polycythemic myelofibrosis phase (post-PV MF) in which cytopenias, including anemia, are associated with ineffective hematopoiesis, bone marrow (BM) fibrosis, extra-medullary hematopoiesis (EMH), and hypersplenism.

Clinical features

The major symptoms of PV are related to hypertension or vascular abnormalities caused by the increased red cell mass. In nearly 20% of patients an episode of venous or arterial thrombosis is documented. Headache, dizziness, visual disturbances and paresthesias are major complaints and gout, pruritus and erythromelalgia are also common. In the full-blown polycythemic stage physical findings include plethora and palpable splenomegaly in 70% and hepatomegaly in 40% of patients.

Prognosis

With currently available treatment, median survival times >10 years are commonly reported. Most patients die from thrombosis or hemorrhage, but up to 20% succumb to myelodysplasia (MDS) or acute myeloid leukemia (AML). The incidence of MDS or AML is only 2-3% in patients who have not been treated with cytotoxic agents, but increases to 10% or more following certain types of chemotherapy.

Post PV MF:

The probability of evolution to PPMF was 16% at 10 years and 34% at 15 years. The actuarial probability of PPMF at 15 years was higher in those patients presenting at diagnosis with

endogenous megakaryocytic colony formation, an elevated serum lactate dehydrogenase (LDH) level, and in those who were heterozygous for the JAK2 V617F mutation.[72]

Primary myelofibrosis (PMF)[79]

PMF is a clonal myeloproliferative neoplasm characterized by a proliferation of predominantly megakaryocytes and granulocytes in the bone marrow (BM) that in fully developed disease is associated with reactive deposition of fibrous connective tissue and with extramedullary hematopoiesis (EMH). There is stepwise evolution from an initial prefibrotic phase characterized by a hypercellular BM with absent or minimal reticulin fibrosis to a fibrotic phase with marked reticulin or collagen fibrosis in the BM and often osteosclerosis. This fibrotic stage of PMF is characterized by leukoerythroblastosis in the blood with tear drop-shaped red cells, and by hepatomegaly and splenomegaly.

The overt fibrotic phase is estimated to occur at 0.5-1.5 per 100,000 persons per year. It occurs most commonly in the sixth to seventh decade of life, and both sexes are nearly equally affected. Children are rarely affected. The etiologic factors responsible for the disease are unknown in the majority of patients. Exposure to benzene or ionizing radiation has been documented in some cases. Rare familial cases of BM fibrosis in young children have been reported.

Clinical features

Up to 30% of patients are asymptomatic at the time of diagnosis and are incidentally discovered to have splenomegaly, anemia, leukocytosis or thrombocytosis. Constitutional symptoms may include fatigue, dyspnoea, weight loss, night sweats and low-grade fever and bleeding disorders. Gouty arthritis and renal stones due to hyperuricemia may also occur.

Prognosis

The time of survival in patients with PMF ranges from months to decades and it depends on the stage in which PMF is first diagnosed. The median survival time is 3-7 years in patients diagnosed in the fibrotic stage, which contrasts with a 10-year relative survival rate of 72% in patients diagnosed in the early prefibrotic phase. Factors at presentation that adversely affect prognosis include age >70 years, Hb <10 gm/dl, platelet count $<100x10^6$ /L and an abnormal karyotype. The major causes of morbidity and mortality are BM failure, thromboembolic events, portal hypertension, cardiac failure and acute leukemia which occurs in 5-30% of patients.

Essential thrombocythemia (ET)[79]

ET is a chronic MPN that involves primarily the megakaryocytic lineage. It is characterized by sustained thrombocytosis $\geq 450x\ 10^9$ /L in the peripheral blood, increased numbers of large, mature megakaryocytes in the BM, and clinically by episodes of thrombosis and/or hemorrhage. Because there is no biologic or genetic marker specific for ET, other causes for thrombocytosis must be excluded, including other MPN, inflammatory and infectious disorders, hemorrhage and other types of hematopoietic and non-hematopoietic neoplasms.

Epidemiology

The true incidence of ET is unknown, but when diagnosed as per the Polycythemia Vera Study Group (PSVG) guidelines, it is estimated to be 0.6-2.5 per 100,000 persons per year. Most cases occur in patients 50-60 years of age with no sex predilection. ET can be infrequently seen in children, but must be distinguished from rare cases hereditary thrombocytosis. The etiology of ET is unknown.

Clinical features

More than 50% of the patients are asymptomatic and are diagnosed incidentally. Others are diagnosed due to thrombotic or hemorrhagic complications. On evaluation few have splenomegaly and 15-20% have hepatomegaly.

Ph-negative MPN in children

MPNs in general, are less frequent in children and the JAK2 V617F mutation in children with PV is significantly less frequent than in adult PV. Children with PV and ET also have a significantly lower incidence of thrombosis than adults.[69]

Thrombosis and bleeding

A complication which is seen in all the three disorders is bleeding or thrombosis. Thrombohemorrhagic complications are a major cause of morbidity and mortality in these disorders.[3] In PV, patients are prone to thrombosis whereas in PMF and ET there are more hemorrhagic problems[11] The pathogenesis of these complications is multifactorial and the relevance of different pathogenetic factors is not completely understood.

A. Incidence of thrombotic events

In PV, fatal and nonfatal arterial and venous thromboses are seen in 41% of patients.[5] Thrombotic events have been documented to occur more frequently in the 2 years preceding diagnosis. This suggests a causal relationship between the latent myeloproliferative disorder and the vascular event. [5] In ET, the incidence of thrombosis and microvascular disturbances or hemorrhage is 8.1% per patient-year and 2.5% per patient-year, respectively.[21]

B. Significance of thrombotic events

Thrombotic events increase the mortality in these diseases.[6] In a large European study on polycythemia (ECLAP trial) the rates of total mortality and mortality from cardiovascular (CV) disease and leukemia were much higher than those expected in the general population (1.2, 1.4, and 36.1 times respectively).[18]

C. Factors associated with thrombosis in Philadelphia-negative myeloproliferative diseases

1. Individual variations: [11]

Some individuals with MPN exhibit a pattern of either exclusively bleeding or thrombotic events; many others have both bleeding and thrombosis during the course of their disease. Furthermore a patient with an MPN may apparently shift from having predominant bleeding complications to being thrombosis prone, or vice versa, as the disease progresses.

A prior history of thrombotic event is a strong predictive factor for new thrombotic events during follow-up. [18]

2. Age and prior thrombotic events[1, 5, 76]:

Increasing age increases the risk of thrombosis in ET and PV

3. Smoking and hypertension[2] :

These are associated with an increased risk of thrombotic events.

4. RBC abnormalities:

Uncontrolled polycythemia: due to increased blood viscosity and the resulting axial migration of RBCs may predispose to thrombosis.[10, 11] In these patients if the hematocrit is corrected to normal by phlebotomy, the major arterial and venous thrombotic complications are reduced.[17]

Biochemical changes in RBCs: Not only a higher hemoglobin, but biochemical changes in the cell membrane and content may also independently impair blood flow. This occurs through the formation of RBC aggregates that have the potential to directly block blood flow in small vessels. This contributes to ischemia and infarction, especially in the cerebral blood flow.[15]

Effect of a high hematocrit on platelet function: Platelet adhesion and thrombus formation also increase as the hematocrit values increase from 10 to 70 percent.[19]

5. Neutrophil (polymorphonuclear cells, PMN) abnormalities:

Leukocytosis: There is a linear correlation between WBC counts and thrombosis (especially myocardial infarction) in both ET and PV.[1, 2, 76]

Activated neutrophils: In ET and PV, a series of PMN activation parameters (PMN membrane CD11b and leukocyte alkaline phosphatase [LAP] antigen expression, cellular elastase content, plasma elastase, and myeloperoxidase levels) have been studied.[10] The interplay between activated PMN and activated platelets generates PMN/platelet-mixed aggregates. These are known to be increased in several pathological conditions associated with a propensity to thrombosis.[15]

6. Platelets

Thrombocytosis: In one analysis the platelet number did not have a significant association with thrombosis in ET.[1] But thrombocytosis may be contributing to the vascular events of ET or PV, as suggested by the evidence that platelet count reduction lowers the risk of microcirculatory disturbances.[15] On the other hand, marked thrombocytosis could favor hemorrhagic rather than thrombotic manifestations in ET patients. [15]

Functional platelet abnormalities: Platelets in thrombocythemia (ET and PV) are hypersensitive. Due to the existing high shear stress in the microvasculature (end-arterial circulation), platelets spontaneously activate and secrete their products. They form aggregates mediated by von Willebrand factor (vWF). These transiently plug the microcirculation, deaggregate, and then recirculate as exhausted defective platelets with secondary storage pool disease on ex vivo analysis. Thus platelets in these conditions are in a state of activation. At increasing platelet counts from below to above 1000 x 10 (9)/l, the thrombotic condition changes into an overt spontaneous bleeding tendency as a result of a functional vWF deficiency that is caused by proteolysis of large vWF multimers.[11, 31] These events are due to functional abnormalities in these disorders. These complications are seen in patients with reactive or secondary polycythemia and thrombocytosis. But in polycythemia vera, essential thrombocytosis and idiopathic myelofibrosis these are probably an effect of the abnormal clone of cells. Especially since some features like erythromelalgia never occurs in secondary thrombocytosis[15]

Platelet microparticles (PMP): After activation of platelets with certain stimuli, there is a release of vesicular particles called platelet microparticles. These microparticles have been shown to

accelerate thrombin generation.[62] Patients with a history of venous or arterial thrombotic events have been shown to have more circulating microparticles than patients without thrombosis.[50]

7. Endothelial damage:

In ET and PV increased levels of thrombomodulin and von Willebrand factor antigen have been found. This suggests that there is a contribution from endothelial damage to thrombosis.[10] Although, the mechanism of endothelial damage is not clear.

8. Thrombophilia:

The contribution of thrombophilic states to the thrombosis in Philadelphia-negative MPN has been documented in some studies. A carrier state for FV Leiden has been associated with venous thromboembolism (VTE) relapse.[25] High factor V levels, anti-phospholipid antibody (APLA)[49] and acquired activated protein C resistance (aAPCR)[51, 52] are also independently associated with an increased risk of thrombosis.[44]

9. Coagulation system activation:

Probably due to a combination of the above-mentioned factors, there is activation of the coagulation system as a whole. This has been shown by increased serum thrombin-antithrombin complex, prothrombin fragment 1 + 2, and D-dimer in ET and PV.[10]

10. Association between the $JAK2^{V617F}$ mutation and thrombosis in Philadelphia negative MPN

JAK2 (Janus kinase 2) is a cytoplasmic tyrosine kinase with a key role in signal transduction from multiple hemopoietic growth-factor receptors.[7] The gene which encodes the JAK2 protein is present on chromosome 9p24.

JAK2^{V617F} mutation

A clonal and recurrent mutation in the JH2 pseudo-kinase domain of the Janus kinase 2 (*JAK2*) gene is seen in several patients with Philadelphia negative myeloproliferative diseases. The mutation, a valine-to-phenylalanine substitution at amino acid position 617, leads to constitutive tyrosine phosphorylation activity that promotes cytokine hypersensitivity.[8]

The prevalence of JAK2 mutation is as given in table 1[7].

Table 1: Prevalence of JAK2 positivity

	JAK2 positive
Polycythemia vera	97%
Essential thrombocythemia	57%
Idiopathic myelofibrosis	50%

The expression of JAK2V617F in cytokine dependent cell lines confers cytokine independence. There is cytokine hypersensitivity too. These occur through the constitutive activation of STAT5, Akt and ERK-dependent pathways.[59] This triggers terminal erythroid amplification in cells from patients with polycythemia vera.[58]

JAK2-V617F stem cells show signs of senescence and exhaustion.[67] *JAK2*-V617F has been reported to induce DNA damage, and has been shown to increase homologous recombination and genetic instability.[68]

JAK2 mutation and latent MPN

The JAK2-V617F mutation is present even in patients who do not have overt features of MPN. Thus this mutation is able to diagnoses patients with latent MPN, most often ET. In 38% of patients with splanchnic vein thrombosis (SVT) the JAK2 mutation is detected even though they have normal/low blood cell counts. In these patients, polycythemia and thrombocytosis may be present. Overt clinical features of PV or ET are masked by hemodilution and/or hypersplenism in these patients, making the diagnosis of MPN difficult.[4, 29]

JAK2 mutation and blast transformation

JAK2 mutation has not been shown to be maintained in the blasts, after blast transformation.[63]

Influence of the JAK2 mutation on thrombotic tendency

JAK2 and hemostatic activation variables:

Patients with ET with the JAK2 mutation have significantly lower levels of free protein S (PS) and higher levels of tissue factor (TF), soluble P-selectin,[26, 28] soluble CD40 ligand (sCD40L), von Willebrand antigen (VWF:Ag), surface thrombomodulin (sTM) and plasma thrombomodulin[10] than those with the wild-type allele.[30]

Circulating platelet/PMN aggregates were significantly greater in the JAK2-mutation carriers than in the wild-type and controls.

PMN surface activation and inflammatory markers (i.e., CD14, TF, CD11b, and leukocyte alkaline phosphatase [LAP]) are all significantly higher in ET versus control subjects, with CD14 and LAP being the highest in the JAK2 mutation carriers.

Thus various biochemical markers of a prothrombotic state are elevated in JAK2 mutation carriers.

Patients with ET wild-type, ET V617F, and PV have shown a rate of thrombosis of 1.4%, 2.1%, and 2.7%/patients/year, respectively. In PV this was found to progressively increase according to time of diagnosis. Actuarial probability of arterial and venous thrombosis in the first 5 years of diagnosis was roughly similar in the three groups. While in the subsequent periods, the curves of mutated ET patients diverged from wild-type, and after 10 to 15 years the ET-mutated arm approached PV. These findings may support the concept of a continuum between ET JAK2 mutated and PV, not only in reference to the hematological phenotype, but also in terms of vascular events. [66]

A plausible explanation for these conflicting results may be that most of the studies are on patients with ET. These studies had a relatively small number of mutated patients ranging from 38 to 165. Also fewer patients with ET are JAK2 mutation positive as compared to polycythemia vera. Compounding this issue is the fact the not all the patients which are studied will have thrombosis or bleeding. Thus these studies had a small study sample size with only about 10 to 42 patients suffering from major cardiovascular events. [59]

Bleeding in Philadelphia negative MPN[18]

A positive history of bleeding was present in 8.1% of patients of PV in the ECLAP trial.

The factors associated with bleeding are:

- Age
- Disease duration

- Prior history of bleeding
- High platelet counts (>1000 x 10^9/L)[77]

Blast transformation

The presence of *JAK2V617F* appears not to be a prerequisite for leukemic transformation of MPN, and additional genetic events seem to be required for full transformation. *JAK2V617F* in association with 9p copy number neutral-loss of heterozygosity (CNN-LOH) appear to have no impact on the time to MPN transformation. Nevertheless, the homozygous driver mutation in combination with additional newly acquired aberrations in terms of a second hit may have an implication on the clinical course of MPN-blast phase patients. Since, homozygous *JAK2* mutation has been linked to an inferior outcome in MPN-blast crisis in comparison with patients with either heterozygous *JAK2V617F* or wild-type *JAK2*.[64]

Patients who are JAK2 positive but do not fulfill criteria for MPN

Some patients may fulfill criteria for MPN on follow-up. The presence of JAK2(V617F) mutation should been suggested a prothrombotic state for cerebral, coronary and peripheral microvascular disturbances and for splanchnic vein thrombosis but not for deep vein thrombosis.[74]

Treatment

The treatment of patients varied as per the diagnosis.

<u>Polycythemia vera</u>

Patients of PV had usually been treated with venesection to maintain a hematocrit of 45%. Hydroxyurea was used if this target was not reached with regular venesection or in high risk patients. Other indications for Hydroxyurea were symptomatic or progressive splenomegaly, platelet counts >1500x10^9 /L and progressive leukocytosis. Risk was determined by the presence of a previous history of thrombosis or by age >60 years. Low-dose Aspirin was given to all patients.

Essential thrombocythemia

All patients were treated with Aspirin, except if the platelet counts were >1000x 10^9 /L, when this was first reduced with Hydroxyurea. All patients who were at a high risk of thrombosis are treated with Hydroxyurea.[76]

Primary myelofibrosis

PMF was treated with Hydroxyurea, or with Thalidomide with or without Prednisolone. Hydroxyurea was used for symptomatic splenomegaly and for those who had symptomatic thrombocytosis and/or leukocytosis. Splenomegaly and cytopenias also respond to Thalidomide with or without low-dose Prednisolone. Aspirin was given along with Thalidomide.

Aspirin is often given to patients with MPN to mitigate the risk of thrombosis. Low-dose aspirin administered once daily is usually given but there is some data to suggest that this may be inadequate to fully suppress thromboxane production in ET, since accelerated platelet regeneration in most aspirin-treated ET patients may explain aspirin-persistent TXA_2 biosynthesis through enhanced COX-2 activity and faster renewal of unacetylated COX-1.[70]

OBJECTIVES AND METHODS

Objectives and aims of study

1. To study the clinical profile of patients with Philadelphia negative chronic myeloproliferative diseases.
2. To assess the prevalence of thrombotic events in these disorders in our population.

Hypotheses

The phenotype of the classical Philadelphia negative chronic myeloproliferative disease in India is similar to that reported in the International literature.

Inclusion Criteria (All)

1. All the patients who have undergone testing for JAK2 mutation in Christian Medical College, Vellore.
2. All of these patients who can be categorized in to a specific Philadelphia negative myeloproliferative neoplasm diagnostic category, as described in the 'WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues', Fourth Edition. An outline of this has been mentioned in the chart.
3. Patients who have been inadequately evaluated but nevertheless have some features of MPN and hence can be labeled as 'probable MPN'.

Myeloproliferative Neoplasm	Criteria
Polycythemia vera*	
Major criteria	Hemoglobin > 18.5 g/dL in men or 16.5 g/dL in women or other evidence of increased RBC volume; presence of *JAK2*V617F or other functionally similar mutation such as *JAK2* exon 12 mutation
Minor criteria	Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation; serum erythropoietin level below the reference range for normal; endogenous erythroid colony formation in vitro
Essential thrombocythemia†	Sustained platelet count ≥ 450 × 10^9/L; bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes; no significant increase or left shift of neutrophil granulopoiesis or erythropoiesis; not meeting WHO criteria for polycythemia vera, primary myelofibrosis, BCR-ABL1–positive chronic myelogenous leukemia, myelodysplastic syndrome, or other myeloid neoplasm; demonstration of *JAK2*V617F or other clonal marker, or in the absence of *JAK2*V617F, no evidence for reactive thrombocytosis
Primary myelofibrosis‡	
Major criteria	Presence of megakaryocyte proliferation and atypia, usually accompanied by either reticulin and/or collagen fibrosis, or in absence of significant reticulin fibrosis, a prefibrotic cellular-phase disease; not meeting WHO criteria for polycythemia vera, BCR-ABL1–positive chronic myelogenous leukemia, myelodysplastic syndrome, or other myeloid neoplasm; demonstration of *JAK2*V617F or other clonal marker (eg, *MPL*W515K/L), or in absence of clonal marker, no evidence of secondary bone marrow fibrosis
Minor criteria	Leukoerythroblastosis; increase in serum lactate dehydrogenase level; anemia; splenomegaly

*Diagnosis requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria.
†Diagnosis requires meeting all four criteria.
‡Diagnosis requires meeting all three major and two minor criteria.

WHO criteria for diagnosis of polycythemia vera, essential thrombocythemia and primary myelofibrosis

Exclusion Criteria (Any)

1. Patients who haven't been evaluated adequately so as to be able to categorize them in to any specific diagnosis of Philadelphia negative myeloproliferative neoplasm.
2. Philadelphia chromosome positivity by conventional cytogenetics, FISH or RT PCR.

Study design

1. A retrospective cross sectional study of patients who are diagnosed to have the classical Philadelphia-negative chronic myeloproliferative disease (polycythemia vera, essential thrombocytosis, idiopathic myelofibrosis) and probable MPN. From June 2009 the patient data was also followed prospectively till August 2010.
2. The analysis included all the patients with these diagnoses, who were seen in the Department of Hematology of Christian Medical College, Vellore between January 2005 and September 2010.

3. They were assessed for their clinical presentation, which included the history and clinical findings.
4. Their laboratory features were documented with respect to the hematologic parameters, JAK2 mutation status.

Patients

The database of patients who had undergone testing for JAK2 mutation was searched for identifying patients with MPN. Outpatient and inpatient records of these patients were accessed from the Medical Records Department. All efforts were taken to enroll as many patients as possible. Consecutive patients who had undergone testing for JAK2 mutation and for whom medical records were available were enrolled in the study to prevent any recruitment bias.

The clinical features at presentation including the signs and symptoms were recorded. The details of the laboratory findings at diagnosis and on follow-up were noted too. If the patients had undergone initial evaluation at another medical facility, then the clinical findings and laboratory values recorded at that time were used for analysis.

Thrombosis was defined any thrombotic event affecting the arterial circulation leading to decreased organ perfusion such as cerebral or coronary ischemia or occlusion of the venous circulation leading to deep vein, abdominal vein thrombosis, etc. Clinical symptoms of erythromelalgia, transient ischemic attack (TIA) and tingling or numbness of extremities, which are due to microvascular insufficiency were categorized as microvascular events.

Laboratory evaluation

Patients had undergone a complete blood count on Beckman Coulter LH 750 cell counter. Peripheral blood smears were reviewed if the cell counter flagged for high or low values of hemoglobin, WBC or platelets or if immature WBCs were identified by the cell counter. Peripheral smears were stained by Leishman stain and a manual differential WBC count was done on 100 cells. Liver and renal function tests and lactate dehydrogenase (LDH) were tested on automated chemistry analyzers. Some patients with thrombosis had undergone evaluation for a prothrombotic condition.

Bone Marrow Aspirate

Bone marrow was aspiration and core biopsy were done as part of diagnostic evaluation from the posterior superior iliac spine. The bone marrow aspirate smears were stained with May Grunwald Geimsa stain and a differential count was done on 500 nucleated cells. If immature cells were identified on the bone marrow aspirate smear then cytochemistry was performed using Sudan Black B and Periodic acid Schiff reagent stains. Flow cytometry for was done for abnormal cells if the bone marrow morphology and cytochemistry was not diagnostic. The bone marrow trephine was fixed in paraffin by the slow decalcification method. Staining was with hematoxylin and eosin (H&E) stain for general architecture and morphology and Gomori silver stain for reticulin.

JAK2 mutation analysis

The JAK2 mutation was detected with an allele specific PCR for detecting V617F mutation in Exon 14 of the Jak2 gene as follows: [7]

9 ml of EDTA anti coagulated peripheral blood was collected and stored at 4C until DNA extraction. DNA was extracted using Phenol-Chloroform method and stored at 4C. 100ng of patients DNA was amplified in a 36-cycle PCR reaction at an annealing temperature of 58°C. 1 micromolar concentration of a common reverse primer and 05 micromolar concentration of two forward primers will used as given below

Reverse: 5-CTGAATAGTCCTACAGTGTTTTCAGTTTCA-3

Forward (specific): 5-AGCATTTGGTTTTAAATTATGGAGTATATT-3

Forward (internal control): 5-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3

The first forward primer is specific for the mutant allele and contains an intentional mismatch at the third nucleotide from the 3 prime end to improve specificity (giving a 203-bp product); the second amplifies a 364-bp product from both mutant and wild-type alleles and serves as an internal PCR control. The PCR products will then be run on a 3% agarose gel. Presence of the low molecular weight (203-bp) band indicates the mutation is carried by the patient; the high molecular weight (364-bp) band acts as an internal PCR control.

Treatment

Patients had been treated as per the outlines mentioned in the chart given below.

Definitions

Response criteria

The responses to treatment for patients with PMF were graded as per the recommendations of the International working group[80] and that for PV and ET as per the European LeukemiaNet consensus conference.[81]

Statistical analysis

Descriptive statistics were calculated for all variables. Statistical analysis was performed using SPSS 16.0 software (SPSS,Chicago, IL).

RESULTS

RESULTS

A total of 540 patients underwent testing for JAK2 mutation in CMC, Vellore from January 2005 to September 2010. Charts, for review, were available for 477 patients. Out of these, an analysis was done on 227 patients as mentioned in figure 1.

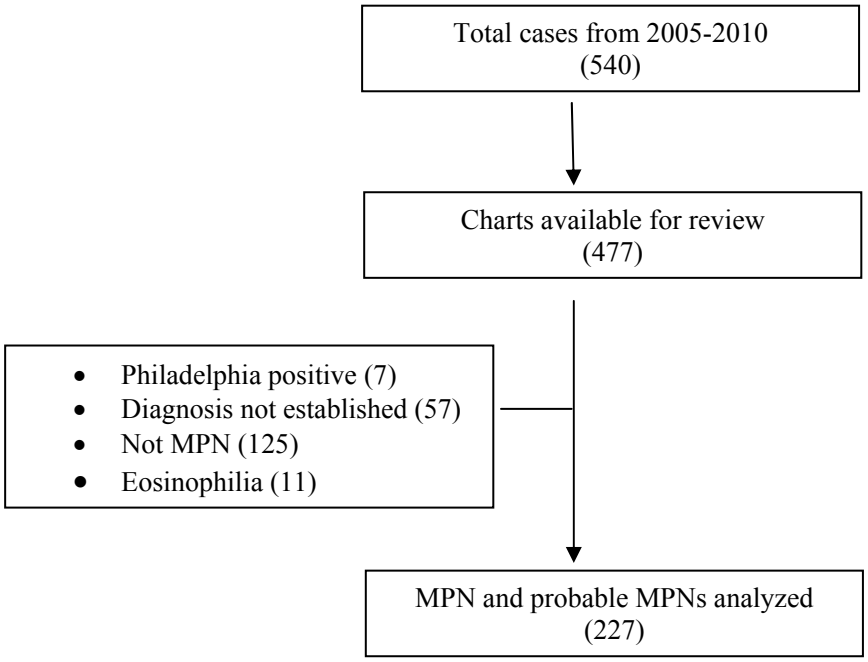


Figure 1: Flow chart depicting the patient selection.

Baseline Characteristics

The most common diagnosis was polycythemia, either polycythemia vera or secondary erythrocytosis, 26.3% and 29% respectively. Details of the frequency of various diagnoses have been given in table 2. Patients with polycythemia vera, essential thrombocythemia and primary myelofibrosis, cellular phase of myelofibrosis, post PV and post ET myelofibrosis constituted the majority of patients. Patients with polycythemia who were JAK2 negative, with a high

erythropoietin level and whose bone marrow features were not suggestive of polycythemia vera, as per the WHO criteria, were classified as secondary polycythemia. Some patients had undergone JAK2 mutation analysis after having presented with abdominal vein thrombosis. A subset of these did not have any clinical or laboratory evidence of having a myeloproliferative neoplasm and these were categorized as patients with isolated abdominal vein thrombosis without any obvious underlying MPN. Patients with secondary polycythemia and those with abdominal vein thrombosis without any other evidence of MPN were not included in the further analysis since they did not fulfill the inclusion and exclusion criteria. A significant subset of patients had some clinical and laboratory evidence of a myeloproliferative neoplasm, but they had not undergone sufficient diagnostic evaluation to categorize them in to a specific type of MPN. These patients were included in the analysis as a 'probable MPN' group.

Table 2: Frequency of diagnosis

Diagnosis	N	Percent
PV	96	26.3
ET	31	8.5
PMF	48	13.2
Cellular phase of PMF	18	4.9
Secondary polycythemia	106	29
Abdominal vein thrombosis (AVT)	32	8.8
Post ET MF	1	0.3
Post PV MF	5	1.4
Probable MPN	28	7.6

From the 277 patients who were further analyzed, 179 (78.9%) were males and 48 (21.1%) were females (table 3A). There was a male predominance in all the diagnostic subsets, except in ET (table 3A). The mean age of diagnosis was 47.8 (±12.5) years, with the range being 10-75 years. The youngest patient was 10 years old and had ET.

The other baseline characteristics which could potentially contribute to an increased risk of thrombosis have been detailed in table 3B. A large proportion of patients with polycythemia were hypertensive, 44 (45.8%) and diabetic 18 (18.8%).

Table 3A: Age and sex frequencies

Diagnosis	Age (years) Mean ± SD	Sex	
		Male n (%)	Female n (%)
Overall	47.8 ±12.5	179 (78.9)	48 (21.1)
PV	48.68 ±11.44	83 (86.5)	13 (13.5)
ET	41.55 ±13.57	16 (51.6)	15 (48.4)
PMF	48.54 ±12.62	36 (75)	12 (25)
Cellular phase of PMF	50.17 ±14.23	15 (83.3)	3 (16.7)
Post ET MF	36	1 (100)	0 (0)
Post PV MF	59.2 ±6.02	4 (80)	1 (20)
Probable MPN	47.04 ±12.35	24 (85.7)	4 (14.3)

Table 3B: Other baseline characteristics

	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post ET MF n (%)	Post PV MF n (%)	Probable MPN n (%)	General population %
Hypertensive	44 (45.8)	6 (19.4)	10 (20.8)	3 (16.7)	0 (0)	2 (40)	14 (50)	4-45%[107]
Diabetic	18 (18.8)	3 (9.7)	5 (10.4)	1 (5.6)	0 (0)	1 (20)	3 (10.7)	3-16%[107]
Smoking	19 (19.8)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	10 (35.7)	10-45%[107]
Alcohol	7 (7.3)	0 (0)	1 (2.1)	0 (0)	0 (0)	0 (0)	2 (7.1)	6-60%[108, 109]
Dyslipidemia	9 (9.4)	1 (3.2)	0 (0)	1 (5.6)	0 (0)	0 (0)	2 (7.1)	14-39%[107]
Chronic kidney disease	2 (2.1)	1 (3.2)	2 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0.8-1.4%[110]

Duration of illness, and symptoms, clinical features, laboratory characteristics at diagnosis (table 4A and 4B)

- Hepatomegaly was prominent in PMF median 2 (0-14) cm and post PV MF, median 3 (0-4) cm. However, one patient of PV also had a liver enlargement of 17 cm below the costal margin.
- Similarly, splenomegaly was prominent in PMF, median enlargement 10 (0-23) cm below the costal margin, cellular phase of PMF median 4.5 (0-20) cm and post-PV MF median 16 (1-22) cm.
- As expected, hemoglobin (Hb) was highest in PV, mean 18.8 (±2.29) g/dl. The probable MPN group had an Hb of 18.14 (±3.5) g/dl. The lowest mean Hb was in PMF, mean 10.8 (±3.6) g/dl. The lowest value of Hb was in a patient of PMF, 3.9 g/dl.
- The highest median WBC count was in patients with post PV MF and cellular phase of MF, 40.9x10^3 /cmm (3.8-67.1) and 24.8 x10^3 /cmm (3.5-63.1), respectively. One patient of PMF had a WBC count of 316.4 x10^3 /cmm.
- The highest median platelet counts were in ET (1087 x10^3 /cmm (597-2620)), cellular phase of MF (603.5 x10^3 /cmm (151-3193)) and in the one patient with post-ET MF (1184 x10^3 /cmm). Overall the highest platelet count was in a patient with PMF, 3457 x10^3 /cmm.
- The highest LDH values were in PMF (1584.5 (218-3798) U/l) and post-PV MF (1441.8 (±707) U/l).
- Erythropoietin levels in PV were 3.7 (1.56-29.6).

Salient points in presenting symptoms at diagnosis (table 5)

- Several (n=72, 31.7%) of the patients, such as those with polycythemia (45.7%) and ET (35.5%) had been incidentally detected to have an MPN while being evaluated for an unrelated illness.
- The most common clinical feature by which patients of PV, ET and probable MPN had presented to a doctor was thrombosis.
- Bleeding was present in 9 (4%) of patients.
- Rubor and aquagenic pruritus was the presenting feature in very few patients with polycythemia.
- Erythromelalgia was only present in 4 (1.8%) patients with PV and ET
- Splenomegaly was the commonest presenting feature in both the cellular and fibrotic phase of PMF and was seen in about 30% of these patients.
- Similarly, weight loss was the presenting feature in several patients with myelofibrosis, whether it was PMF, post ET or post PV MF.
- Fever, Raynaud's phenomenon, hyperviscosity, giddiness and tingling of extremities were rare presenting feature.
- Anemia, as a presenting feature was only seen in PMF.

Table 4A: Duration of illness, and symptoms and clinical features at diagnosis (median (range))

Diagnosis	Duration of symptoms (weeks)	Liver (cm)	Spleen (cm)
PV	2 (0-520)	0 (0-17)	0 (0-16)
ET	6 (0-196)	0 (0-4)	0 (0-18)
PMF	8 (0-312)	2 (0-14)	10 (0-23)
Cellular phase of PMF	4 (0-104)	0 (0-7)	4.5 (0-20)
Post ET MF	32	0	0
Post PV MF	32 (12-156)	3 (0-4)	16 (1-22)
Probable MPN	3.5 (0-176)	0 (0-7)	0 (0-30)

Table 4B: Laboratory characteristics at diagnosis

Diagnosis	Hb g/dl	MCV ul	WBC $x10^3$/cmm	Platelets $x10^3$/cmm	LDH U/l	Erythropoietin
	(mean±SD)		median (range)		Mean ±SD or median (range)	median (range)
PV	18.8±2.29	82.5±11.8	13.8 (4.8-49.9)	332.5 (12-1600)	658 ±287	3.7 (1.56-29.6)
ET	12.5±2.14	83.4±10.3	13.6 (4.4-56)	1087 (597-2620)	574 ±182	6.3
PMF	10.8±3.6	85.1 ±9.9	12.8 (2.6-316.4)	292 (5-3457)	1584.5 (218-3798)	NA
Cellular phase of PMF	13.1±3	84.5±12.4	24.8 (3.5-63.1)	603.5 (151-3193)	954.6±485	24.8
Post ET MF	11.2	107.4	6.8	1184	231	NA
Post PV MF	14.9±2.5	73.4±10.6	40.9 (3.8-67.1)	328 (108-752)	1441.8±707	3.34
Probable MPN	18.14±3.5	87.1±15.7	11 (1.8-38.8)	309.5 (43-1228)	467 (235-1557)	8.45 (4-18.5)

Legend: Hb: hemoglobin, MCV: mean corpuscular volume, WBC: white blood cells, LDH: lactate dehydrogenase

Table 5: Symptoms at diagnosis

Clinical manifestation	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post ET MF n (%)	Post PV MF n(%)	Probable MPN n (%)
No symptoms	43 (45.7)	11 (35.5)	6 (13)	3 (16.7)	0 (0)	1 (20)	8 (29.6)
Thrombosis	23 (24.5)	8 (25.8)	0 (0)	3 (16.7)	0 (0)	1 (20)	8 (29.6)
Bleeding	3 (3.2)	0 (0)	1 (2.2)	2 (11.1)	0 (0)	0 (0)	3 (11.1)
Rubor	3 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.7)
Splenomegaly	4 (4.3)	1 (3.2)	14 (30.4)	6 (33.3)	0 (0)	0 (0)	1 (3.7)
Aquagenic pruritus	2 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Weight loss	1 (1.1)	0 (0)	7 (15.2)	2 (11.1)	1 (100)	2 (40)	1 (3.7)
Erythromelalgia	1 (1.1)	3 (9.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Headache	5 (5.3)	4 (12.9)	1 (2.2)	1 (5.6)	1 (20)	(0)	0 (0)
Fever	0 (0)	1 (3.2)	2 (4.3)	1 (5.6)	0 (0)	0 (0)	0 (0)
Raynaud's phenomenon	1 (1.1)	2 (6.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Anemia	0 (0)	0 (0)	7 (15.2)	0 (0)	0 (0)	0 (0)	0 (0)
Tingling of extremities	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (7.4)
Generalized weakness	6 (6.4)	0 (0)	7 (15.2)	0 (0)	0 (0)	0 (0)	3 (11.1)
Hyperviscosity	0 (0)	0 (0)	1 (2.2)	0 (0)	0 (0)	0 (0)	0 (0)
Giddiness	1 (1.1)	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 6: Gout and pruritus during the course of illness

Clinical manifestation	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post ET MF n (%)	Post PV MF n (%)	Probable MPN n (%)
Gout	1 (1)	0 (0)	1 (2.1)	1 (5.6)	1 (100)	1 (20)	1 (3.6)
Pruritus	9 (9.4)	1 (3.2)	2 (4.2)	1 (5.6)	0 (0)	1 (20)	3 (10.7)

Prevalence of gout and pruritus during the course of illness (table 6)

Gout: Present in 6 (2.6 %) of patients, mostly in those with myelofibrosis, table 6.

Pruritus: Was present in 17 (7.5%) of patients and mostly in those with polycythemia (9.4%), table 6.

Vascular events during the course of illness

Arterial (table 7): Arterial thromboses were were most frequent in the probable MPN group where 10 (35.77%) of patients had thrombosis. This was followed by PV and ET where 29 (29.9%) and 8 (25.8) of patients had suffered some thrombotic event, respectively. The details of these have been described subsequently.

1. **Microvascular events (fig. 2):** Erythromelalgia and tingling and numbness of the extremities were the most frequent microvascular event and were seen in only 5 (2.2%) and 1 (0.4%) of patients respectively.

   Visual disturbances suggestive of amaurosis fugax, were present in isolated patients of PV and ET.

   Three (3.1%) patients of PV had Raynaud's phenomenon.

2. **Macrovascular thrombosis (fig. 3)**

   Macrovascular thrombosis was most frequent in PV seen in 22 (22.8%) and in the probable MPN group where 8 (28.6%) of patients had such events.

   Thrombotic events of the central nervous system (CNS), including stroke and TIA, were seen with the maximum frequency in PV, 17 (17.7%).

   7 (3.1%) of patients had coronary arterial thrombotic events.

Gangrene of the extremities was seen in 5 (2.2%) patients. This included brachial artery occlusion in 1 (0.4%), toe gangrene in 3 (1.3%) and lower limb gangrene, not otherwise specified in 1 (0.4%) of patients.

3. **Recurrent:** A second episode of stroke and transient ischemic attack was present in one patient of PV each.

Venous thrombosis (table 8 and figure 4)

Venous thrombosis was most frequent in ET since 7 (22.6%) of patients had such events.

The commonest site was intraabdominal veins in 16 (5.8%) of patients as the site of first thrombosis. In 1 (0.4%) patient an abdominal vein thrombosis (AVT) was the second site of thrombosis. This patient had developed the first episode of thrombosis in the upper limb which was followed by abdominal vein thrombosis.

The other sites of first thrombosis were cortical venous sinus thrombosis (CVT) in 5 (2.2%) and lower limb deep vein thrombosis (DVT) in 3 (1.3%) each. One (0.4%) patient had been diagnosed to have upper limb deep venous thrombosis.

Recurrent venous thrombosis was seen in 2 (0.8%) of patients. One patient had two episodes of lower limb DVT and the other had AVT as described above.

Multiple thromboses (table 9)

Overall, a total of 7 (3.1%) of patients had developed multiple thrombosis. These are detailed in table 9. Six of these seven patients had polycythemia. One patient with multiple thromboses had ET.

Evaluation for a prothrombotic work-up (table 10)

Amongst all the patients who had suffered a thrombotic event, testing for a prothrombotic condition was performed in 8 patients and an abnormality was detected in 4 patients. Their details have been mentioned in table 10.

Figure 2: Microvascular events

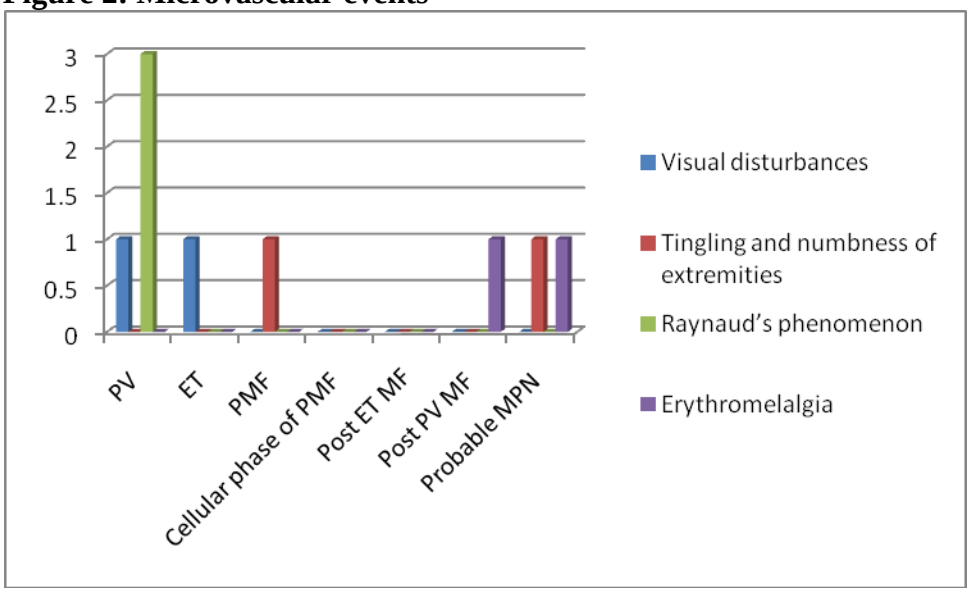
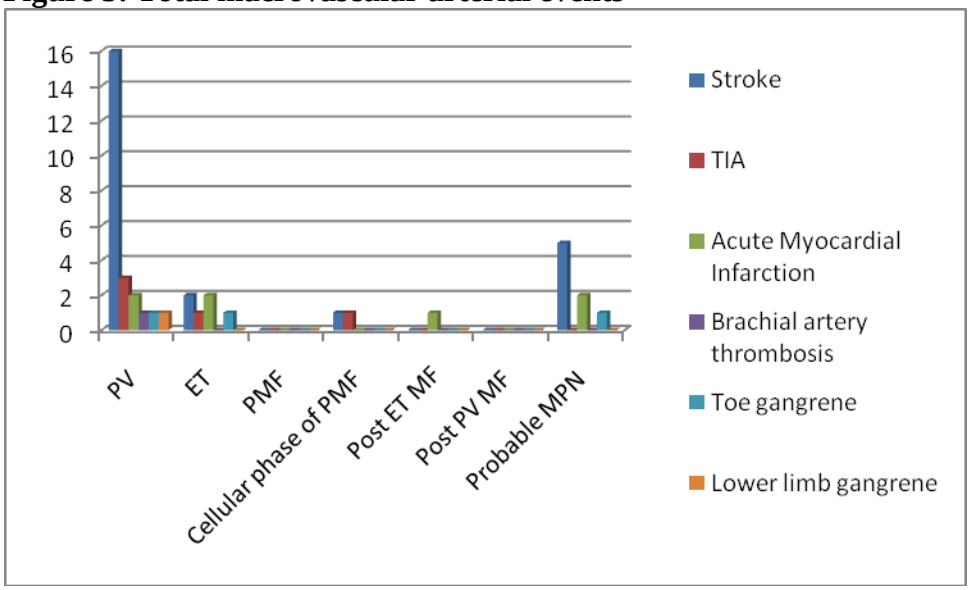
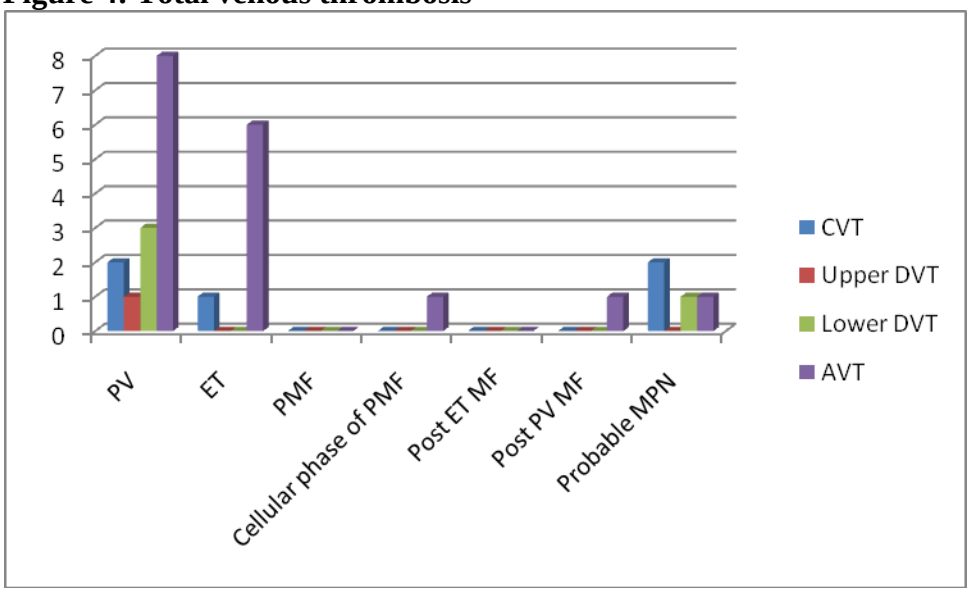


Figure 3: Total macrovascular arterial events



Legend: TIA: transient ischemic attack

Figure 4: Total venous thrombosis



Legend: CVT: cortical venous thrombosis, DVT: deep vein thrombosis, AVT: abdominal vein thrombosis

Figure 5: Frequencies of bleeding events

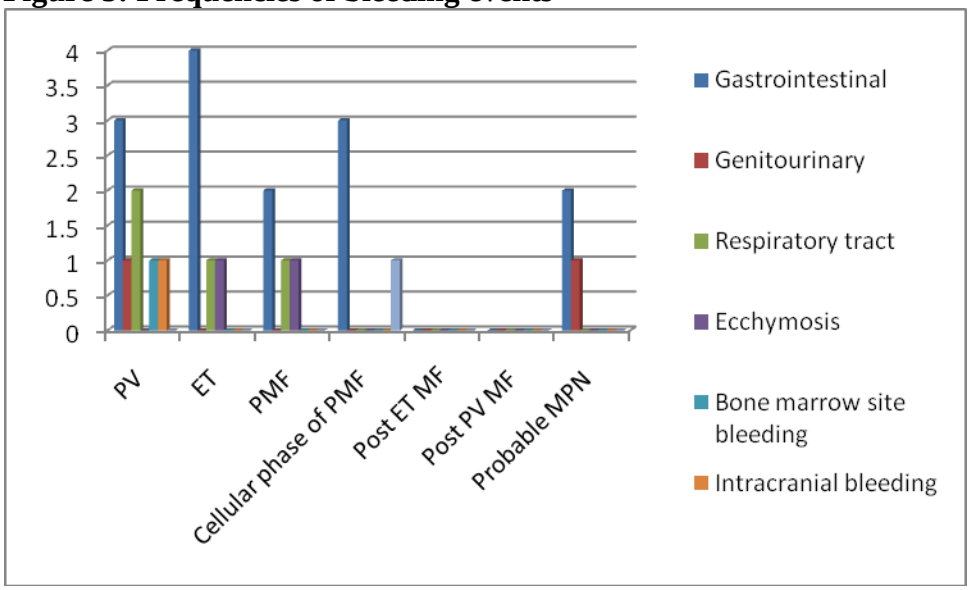


Table 7: Arterial vascular events during the course of illness

Vascular events	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post ET MF n (%)	Post PV MF n (%)	Probable MPN n (%)
Microvascular events							
Visual disturbances	1 (1)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tingling and numbness of extremities	0 (0)	0 (0)	1 (2.1)	0 (0)	0 (0)	0 (0)	1 (3.57)
Raynaud's phenomenon	3 (3.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Erythromelalgia	1 (1)	1 (3.2)	1 (2.1)	0 (0)	0 (0)	1 (20)	1 (3.6)
Total	5 (5.1)	2 (6.4)	2 (4.2)	0 (0)	0 (0)	1 (20)	2 (7.17)
Macrovascular thrombosis 1st event							
Stroke	15 (15.6)	2 (6.5)	0 (0)	1 (5.6)	0 (0)	0 (0)	5 (17.9)
TIA (transient ischemic attack)	2 (2.1)	1 (3.2)	0 (0)	1 (5.6)	0 (0)	0 (0)	0 (0)
Acute Myocardial Infarction	2 (2.1)	2 (6.5)	0 (0)	0 (0)	1 (100)	0 (0)	2 (7.1)
Brachial artery thrombosis	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Toe gangrene	1 (1)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.6)
Lower limb gangrene	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	22 (22.8)	6 (19.4)	0 (0)	2 (11.2)	1 (100)	0 (0)	8 (28.6)
Macrovascular thromboses 2nd event							
Stroke	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
TIA	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Overall total	**29 (29.9)**	**8 (25.8)**	**2 (4.2)**	**2 (11.2)**	**1 (100)**	**1 (20)**	**10(35.77)**

Table 8: Venous thrombosis

Thrombosis	Overall n (%)	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post ET MF n (%)	Post PV MF n (%)	Probable MPN n (%)
CVT	5 (1.8)	2 (2.1)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	2 (7.1)
Upper DVT	1 (0.36)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lower DVT	3 (1.1)	2 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.6)
AVT	16 (5.8)	7 (7.3)	6 (19.4)	0 (0)	1 (5.6)	0 (0)	1 (20)	1 (3.6)
Total	25 (9)	12 (12.5)	7 (22.6)	0 (0)	1 (5.6)	0 (0)	1 (20)	4 (14.3)
Recurrent thrombosis								
Lower DVT	1 (0.36)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
AVT	1 (0.36)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	2 (0.72)	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 9: Multiple thromboses

Patient serial number	Microvascular thrombosis	Macrovascular thrombosis	Venous thrombosis	Diagnosis
70	-	Stroke, TIA	-	PV
88	-	TIA	AVT	ET
91	Raynaud's phenomenon	-	Upper DVT, AVT	PV
110	Erythromelalgia	Toe gangrene	-	PV
140	-	Stroke, stroke (2^{nd} episode)	CVT	PV
156	Raynaud's phenomenon	-	AVT	PV
317	Raynaud's phenomenon	Stroke	-	PV

Table 10: Prothrombotic risk factors

Serial number	Diagnosis	Sites of thrombosis	Prothrombotic risk factor
11	Probable MPN	Lower DVT	High factor VIII
20	Probable MPN	CVT	High factor VIII, lupus anticoagulant
34	Probable MPN	AVT	High factor VIII
284	Cellular phase of PMF	Stroke	lupus anticoagulant

Legend for tables 8-10: CVT: cortical venous thrombosis, DVT: deep vein thrombosis, AVT: abdominal vein thrombosis, TIA: transient ischemic attack

Bleeding (table 11 and figure 5)

The major sites of bleeding were gastrointestinal (GI), genitourinary (GU), respiratory and cutaneous. One patient each had bleeding from the bone marrow biopsy site and intracranial hemorrhage. Similarly, one patient had presented with left knee hemarthrosis of unknown etiology and was diagnosed to have cellular phase of PMF.

GI hemorrhage was the most common site of bleeding and was seen in 14 (6.1%) of patients. This was most frequent in the cellular phase of PMF 3 (16.7%) and ET 4 (12.9) patients.

Genitourinary hemorrhage was seen in one patient of polycythemia and in one patient of probable MPN.

Similarly, occasional patients of PV and ET had epistaxis and one patient of PMF had hemoptysis whose anatomic cause could not be determined.

Cutaneous bleeding in the form of ecchymosis was also present in isolated patients of ET and PMF.

Leukemic transformation (table 12)

In the current analysis 5 (2.2%) patients underwent leukemic transformation. This occurred in 1 (3.2%) of patient with ET and 4 (8.3%) patients with PMF. The patient of ET developed acute myeloid leukemia 367 days after being diagnosed with ET.

Amongst the 4 patients with PMF who had undergone leukemic transformation the median time from diagnosis of PMF to leukemic transformation was 52 weeks (range 33-365).

None of the patients underwent definitive treatment for leukemia and was managed with palliative intent and subsequently expired.

Table 11: Bleeding

Site of bleeding	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post ET MF n (%)	Post PV MF n (%)	Probable MPN n (%)
Gastrointestinal (GI)							
Gum	0 (0)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.6)
Varices	1 (1)	1 (3.2)	1 (2.1)	2 (11.1)	0 (0)	0 (0)	0 (0)
Hemorrhoids	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GI unspecified	1 (1)	2 (6.5)	1 (2.1)	1 (5.6)	0 (0)	0 (0)	1 (3.6)
Total	3 (3)	4 (12.9)	2 (4.2)	3 (16.7)	0 (0)	0 (0)	2 (7.2)
Genitourinary (GU)							
Hematuria	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.6)
GU unspecified	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.6)
Respiratory							
Epistaxis	2 (2.1)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hemoptysis	0 (0)	0 (0)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)
Total	2 (2.1)	1 (3.2)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)
Miscellaneous							
Ecchymosis	0 (0)	1 (3.2)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)
Bone marrow site bleeding	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Intracranial bleeding	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hemarthrosis	0 (0)	0 (0)	0 (0)	1 (5.6)	0 (0)	0 (0)	0 (0)
Overall total	**8 (8.33)**	**6 (19.35)**	**4 (8.33)**	**4(22.22)**	**0 (0)**	**0 (0)**	**3 (10.71)**

Table 12: Details of patients who underwent leukemic transformation

Serial number	Diagnosis	Weeks to leukemic transformation (from diagnosis)	Successive treatments received prior to development of leukemia	Marrow blasts at initial diagnosis of ET/PMF	Lille's score at diagnosis
23	ET	52	• Hydroxyurea	8	-
172	PMF	130	• Hydroxyurea • Interferon alpha	15	0
199	PMF	51	• Thalidomide	<5	1
290	PMF	33	• Thalidomide	<5	1
366	PMF	365	• Hydroxyurea • Thalidomide with Prednisolone	10	0

Table 13: Details of patients who progressed to myelofibrosis

Serial number	Diagnosis	Weeks to leukemic transformation (from diagnosis)	Successive treatments received prior to development of leukemia
96	PV	506	• Venesection • Hydroxyurea
127	Post PV MF	NA	• Venesection • Hydroxyurea
151	PV	NA	• Hydroxyurea • Thalidomide with Prednisolone
242	PV	24	• Venesection
335	PV	1383	• 32Phosphorus • Hydroxyurea • Thalidomide with Prednisolone
339	Post PV MF	112	• Venesection • Hydroxyurea • Thalidomide with Prednisolone
361	PV	537	• Venesection • Hydroxyurea

Table 14: JAK2 positivity

Diagnosis	JAK2 positive n (%)
PV	64 (66.7)
ET	18 (58.1)
PMF	23 (47.9)
Cellular phase of PMF	11 (61.1)
Post-ET MF	1 (100)
Post-PV MF	5 (100)
Probable MPN	12 (42.9)
Total	134 (48.4)

Progression to myelofibrosis, table 13

A total of 7 (7.3%) patients of PV had progression to myelofibrosis and the time for documentation of progression from the time of diagnosis of PV was known in 5 patients. This ranged from 24-1383 weeks with a median of 537 weeks. Two of these patients had been diagnosed outside to have PV and when they came for review to us they had developed progression to myelofibrosis, hence these patients were labeled as post-PV MF.

JAK2 positivity (table 14)

JAK2 mutation was positive in 134 (59%) patients, overall. The highest rate of positivity was in PV, 64 (66.7%). All the patients of post-PV and post-ET MF was JAK2 positive.

Treatment details

These have been described as per the sequential treatments received by patients has been detailed in table 24.

First treatment (tables 15 and 16)

- 32.3% of PV, 80.6% of ET and 83.3% of cellular PMF had been treated with hydroxyurea initially.
- 63.5% of PV received venesection as first line of management.
- Only one (1%) patient of PV had been treated with 32Phosphorus.
- Single agent thalidomide or prednisolone was used in isolated patients of PMF while thalidomide with prednisolone was used in 31.2% of PMF and 5.6%of

cellular PMF patients. Busulfan, splenectomy and splenic irradiation had been given to one patient each of PMF.

- Amongst all the disease groups, whatever treatment was given, 35-80% of patients had achieved a partial remission and clinical improvement in MF.

Second treatment (table 17 and 18)

- Hydroxyurea in 19% and venesection in 25% were the second line treatments for PV.
- For patients with PMF hydroxyurea and thalidomide with prednisolone were used in 8.3% of patients each.
- Similar to the first line of treatment, in almost all the disease groups, whatever treatment was given, 15-80% of patients had achieved a partial remission of stable disease and clinical improvement in MF.

Third and fourth treatments (tables 19-22)

- These were required in very few patients.

Aspirin and oral anticoagulants (table 23)

- Aspirin was used in about 50% of patients.

Table 15: First treatment details

Treatment 1	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post ET MF n (%)	Post PV MF n (%)	Probable MPN n (%)
None	3 (3.1%)	4 (12.9%)	4 (8.3%)	2 (11.1%)	0 (0%)	0 (0%)	5 (17.9%)
Hydroxyurea	31 (32.3%)	25 (80.6%)	22 (45.8%)	15 (83.3%)	1 (100%)	2 (40%)	11 (39.3%)
Venesection	61 (63.5%)	1 (3.2%)	0 (0%)	0 (0%)	0 (0%)	3 (60%)	12 (42.9%)
Thalidomide	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Prednisolone	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Thalidomide + Prednisolone	0 (0%)	0 (0%)	15 (31.2%)	1 (5.6%)	0 (0%)	0 (0%)	0 (0%)
Danazol	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
32Phosphorus	1 (1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Busulfan	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Splenectomy	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Splenic Irradiation	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Transfusions	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stop smoking	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Anagrelide + Hydroxyurea	0 (0%)	1 (3.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 16: Response to first treatment

Response 1	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post ET MF n (%)	Post PV MF n (%)	Probable MPN n (%)
CHCR	23 (24.2%)	4 (14.3%)	1 (2.2%)	2 (12.5%)	0 (0%)	1 (20%)	7 (30.4%)
CR for PMF	NA	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA
PR	68 (71.6%)	21 (75%)	16 (35.6%)	9 (56.2%)	1 (100%)	4 (80%)	14 (60.9%)
PD	NA	NA	6 (13.3%)	1 (6.2%)	0 (0%)	0 (0%)	0 (0%)
SD	NA	NA	14 (31.1%)	1 (6.2%)	0 (0%)	0 (0%)	0 (0%)
NR	0 (0%)	3 (10.7%)	2 (4.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Not known	5 (5.2%)	0 (0%)	6 (13.3%)	3 (18.8%)	0 (0%)	0 (0%)	2 (8.7%)

Legend: CHCR: clinic-hematologic complete remission, for MF this is equivalent to PR by IWG criteria, CR: complete remission, PR: partial remission and is equivalent to clinical improvement (CI) for MF by IWG criteria, PD: progressive disease, SD: stable disease and NR: no response.

Table 17: Second treatment details

Treatment 2	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post PV MF n (%)	Probable MPN n (%)
Hydroxyurea	18 (19%)	0 (0%)	4 (8.3%)	1 (5.6 %)	2 (40%)	2 (7.1%)
Venesection	24 (25 %)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	6 (21.4%)
Thalidomide	0 (0%)	0 (0%)	2 (4.2%)	1 (5.6%)	0 (0%)	0 (0%)
Prednisolone	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Thalidomide +Prednisolone	0 (0%)	0 (0%)	4 (8.3%)	0 (0%)	1 (20%)	0 (0%)
Lenalidomide	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)	0 (0%)
SCT	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)	0 (0%)
32Phosphorus	0 (0%)	1 (3.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
6Mercaptopurine	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)
Transfusions	0 (0%)	1 (3.2%)	1 (2.1%)	0 (0%)	0 (0%)	0 (0%)
Stop smoking	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Legend: SCT: stem cell transplantation

Table 18: Response to second treatment

Response 2	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post PV MF n (%)	Probable MPN n (%)
CHCR	11 (26.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (50%)
CR	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
PR	28 (66.7%)	0 (0%)	2 (15.4%)	2 (100%)	4 (80%)	4 (50%)
PD	0 (0%)	1 (50%)	2 (15.4%)	0 (0%)	0 (0%)	0 (0%)
SD	1 (2.4%)	0 (0%)	4 (30.8%)	0 (0%)	0 (0%)	0 (0%)
NR	0 (0%)	1 (50%)	1 (7.7%)	0 (0%)	0 (0%)	0 (0%)
Not known	1 (2.4%)	0 (0%)	4 (30.8%)	0 (0%)	1 (20%)	0 (0%)

Table 19: Third treatment details

Treatment 3	PV n (%)	PMF n (%)	Post PV MF n (%)	Probable MPN n (%)
Hydroxyurea	1 (1.1%)	1 (2.1%)	2 (40%)	0 (0%)
Thalidomide +Prednisolone	1 (1.1%)	3 (6.3%)	1 (20%)	0 (0%)
Interferon alpha	0 (0%)	1 (2.1%)	0 (0%)	1 (3.6%)
Erythropoietin	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)

Table 20: Response to third treatment

Response 3	PV n (%)	PMF n (%)	Post PV MF n (%)	Probable MPN n (%)
CHCR	0 (0%)	0 (0%)	1 (33.3%)	0 (0%)
PR	2 (100%)	1 (16.7%)	1 (33.3%)	1 (100%)
PD	0 (0%)	2 (33.3%)	1 (33.3%)	0 (0%)
SD	0 (0%)	1 (16.7%)	0 (0%)	0 (0%)
NR	0 (0%)	2 (33.3%)	0 (0%)	0 (0%)

Legend for table 18 and 20: CHCR: clinic-hematologic complete remission, for MF this is equivalent to PR by IWG criteria, CR: complete remission, PR: partial remission and is equivalent to clinical improvement (CI) for MF by IWG criteria, PD: progressive disease, SD: stable disease and NR: no response.

Table 21: Fourth treatment details

Treatment 4	PV n (%)	PMF n (%)
Hydroxyurea	0 (0%)	1 (2.1%)
Thalidomide +Prednisolone	1 (1%)	0 (0%)
Stem cell transplant	(%)	1 (2.1%)
Daflazacort	(%)	1 (2.1%)

Table 22: Response to fourth treatment

Response 4	PVn (%)	PMF n (%)
Partial response	1 (100%)	1 (33.3%)
Progressive disease	0 (0%)	1 (33.3%)
Not known	0 (0%)	1 (33.3%)

Table 23: Patients receiving Aspirin and oral anticoagulants

	PV n (%)	ET n (%)	PMF n (%)	Cellular phase of PMF n (%)	Post ET MF n (%)	Post PV MF n (%)	Probable MPN n (%)	Total n (%)
Aspirin	63 (65.6%)	15 (48.4%)	23 (47.9%)	9 (50%)	1 (100%)	3 (60%)	13 (46.4%)	176 (48.2%)
Oral anticoagulants	8 (8.3%)	5 (16.1%)	0 (0%)	1 (5.6%)	0 (0%)	0 (0%)	3 (10.7%)	53 (14.5%)

DISCUSSION

DISCUSSION

The myeloproliferative neoplasms are a heterogeneous group of diseases. There is minimal data from India regarding the clinical profile and treatment outcome in these disorders.

Diagnostic subsets

During the analysis of 227 patients we found that polycythemia was the commonest MPN. This was followed by PMF and ET which were less frequent. There were several patients who had some clinical features of MPN but who had not undergone complete evaluation to satisfy all the WHO diagnostic criteria. These could not be classified in to any one diagnostic subset and hence were grouped as probable MPN.

Demographic data

The mean age of PV, ET and PMF was about one decade less than what has been reported (table 3A and 25).[79] The youngest patient was 10 years old and had ET.

Table 25: Age distribution among MPNs

	Reported[79]	Our study
PV	60 years	48.68 (±11.44)
ET	6^{th}-7^{th} decade	41.55 (±13.57)
PMF	50-60 years	48.54 (±12.62)

Along with this there was a significant male preponderance in the PV and PMF group (table 3A), although the MPNs have been reported to have an almost equal sex distribution.[79] This discrepancy may be due to lesser availability of medical attention to women in India.[90, 91] Compared to the other disease subsets, a large proportion of patients

with polycythemia were hypertensive, 44 (45.8%) and diabetic, 18 (18.8%), (table 3B). This also seems to be at least slightly more than what is prevalent in the population, although population estimates are heterogeneous and depend on the age group studied. The increased prevalence of hypertension with higher hematocrit is probably related to increased blood viscosity and has been shown to contribute to cardiovascular disease risk.[92-95] 19.8% of patients with PV were smokers.

Duration of illness, and symptoms, clinical features, laboratory characteristics at diagnosis (table 4, 5 and 6)

The duration of symptoms prior to seeking medical attention was varying and was longest in post ET and PV PMF (32 weeks). Several patients, such as those with primary (45.7%) and ET (35.5%) had been incidentally detected to have an MPN while being evaluated for an unrelated illness. This was much higher than what was published by Landolfi, et al who had 16% of patients who had been incidentally detected but these had been diagnosed using the Polycythemia Vera Study Group protocol.[97] For ET, however, incidental diagnosis has been reported in about 50% of patients.[98] Overall, the most common clinical feature by which patients had presented to a doctor was thrombosis. This has been reported earlier, such that in PV up to 2/3rd of the patients have had a thrombotic event before or during presentation with PV.[5]

The second most common presenting manifestation in these patients was splenomegaly. However, this was the commonest presenting feature in both the cellular and fibrotic phase of PMF and was seen in about 30% of these patients.

Bleeding was the presenting symptom in a few patients, especially in PMF (13.3%) similar to what has been described by Varki, et al (20%).[99] Rubor and aquagenic pruritus was the presenting feature in very few patients with either polycythemia. In the series by Seigel, et al, aquagenic pruritus had led to the diagnosis of PV in 4% of patients.[101] Pruritus was present in 27 (11.9%) of patients and mostly in those with polycythemia (9.4%), table 6. This was much lower than what has been reported (50%).[103] The lower prevalence of pruritus in our series may be due to the retrospective nature of the analysis, due to recall bias.

Erythromelalgia was infrequent and was present in only 4 (1.8%) patients with PV and ET, concordant with the published literature.[100] Weight loss was the presenting feature in some patients with myelofibrosis, whether it was PMF (15.2%), post ET or post PV MF. This was similar to what was described by Silverstein.[102] Fever, Raynaud's phenomenon, hyperviscosity, giddiness and tingling of extremities were rare presenting feature. Similarly, anemia, as a presenting feature was only seen in PMF. Gout was present in 6 (2.6%) of patients, mostly in those with myelofibrosis 4 (5.6%), table 6 as described by Silverstein.[102]

Hepatomegaly was prominent in PMF median 2 (0-14) cm and post PV MF, median 3 (0-4) cm. However, one patient of PV also had a liver enlargement of 17 cm below the costal margin. Similarly, splenomegaly was prominent in PMF, with a median enlargement of 10 (0-23) cm below the costal margin, cellular phase of PMF median 4.5 (0-20) cm and post-PV MF median 16 (1-22) cm.

As expected, hemoglobin (Hb) was highest in PV, mean 18.8 (±2.29) g/dl. The probable MPN group had an Hb of 18.14 ±3.5 g/dl. The lowest mean Hb was in PMF, mean

10.8±3.6 g/dl. The lowest value of Hb was in a patient of PMF, 3.9 g/dl. The highest median WBC count was in patients with post PV MF and cellular phase of MF, 40.9x10^3 /cmm (3.8-67.1) and 24.8 x10^3 /cmm (3.5-63.1), respectively. One patient of PMF had a WBC count of 316.4 x10^3 /cmm. The highest median platelet counts were in ET (1087 x10^3 /cmm (597-2620)), cellular phase of MF (603.5 x10^3 /cmm (151-3193)) and in the one patient with post-ET MF (1184 x10^3 /cmm). Overall the highest platelet count was in a patient with PMF, 3457 x10^3 /cmm.

The highest LDH values were in PMF (1584.5 (218-3798) U/l) and post-PV MF (1441.8 (±707) U/l). A serum LDH above the normal range has been shown to occur in 89% of patients with PMF and a serum LDH of twice the upper limit of normal was identified as a reasonable discriminator of PMF from ET/PV, with a sensitivity of 55% and specificity of 94%.[96] Erythropoietin levels in PV were 3.7 (1.56-29.6). Thus these were high in some patients who otherwise were fitting in to the WHO criteria for the diagnosis of PV. These observations are consistent with data which has been published earlier.[104]

Thrombosis during the course of illness

Arterial (table 7): Arterial thromboses were frequent and these were most frequent in the probable MPN group where 10 (35.77%) of patients had thrombosis. This was followed by PV and ET where 29 (29.9%) and 8 (25.8) of patients had suffered some thrombotic event, respectively. The details of these have been described subsequently.

1. **Microvascular events**

Erythromelalgia and tingling and numbness of the extremities were the most frequent microvascular event and were seen in only 5 (2.2 %) and 2 (0.9%) patients respectively.

Visual disturbances suggestive of amaurosis fugax, were present in isolated patients of PV and ET. Three (3.1%) patients of PV had Raynaud's phenomenon.

2. **Macrovascular thrombosis**

Macrovascular thrombosis was most frequent in PV seen in 22 (22.8%) and in the probable MPN group where 8 (28.6%) of patients had such events. Thrombotic events of the central nervous system (CNS), including stroke and TIA, were seen with the maximum frequency in PV, 17 (17.7%). Gangrene of the extremities was seen in 4 (1.8%) patients. This included brachial artery occlusion in 1 (0.4%), toe gangrene in 3 (1.3%) and lower limb gangrene, not otherwise specified in 1 (0.4%) of patients. The prevalence of arterial thrombosis and the observation that the central nervous system was the commonest site of thrombosis is consistent with earlier publications.[104]

3. **Recurrent arterial thromboses**

A second episode of stroke and transient ischemic attack was present in one patient of PV each.

Venous thrombosis (table 8)

Venous thrombosis was most frequent in ET since 7 (22.6%) of patients had such events. The commonest site was intraabdominal veins in 16 (7%) of patients as the site of first thrombosis. In 1 (0.4%) patient an abdominal vein thrombosis (AVT) was the second site of thrombosis. This patient had developed the first episode of thrombosis in the upper limb which was followed by abdominal vein thrombosis. The other sites of first thrombosis were cortical venous sinus thrombosis (CVT) in 5 (2.2 %) and lower limb

deep vein thrombosis (DVT) in 3 (0.88%) each. One (0.4%) patient had been diagnosed to have upper limb deep venous thrombosis.

Recurrent venous thrombosis was seen in 2 (0.8%) of patients. One patient had two episodes of lower limb DVT and the other had AVT as described above. Similar to arterial thrombosis, the prevalence of venous thrombosis is also consistent with what has been published earlier.[104]

Multiple thromboses (table 9)

Overall, a total of 7 (3.1%) of patients had developed multiple thrombosis. These are detailed in table 9. Six of these seven patients had polycythemia. One patient with multiple thromboses had ET.

Evaluation for a prothrombotic work-up (table 10)

Amongst all the patients who had suffered a thrombotic event, an evaluation for a prothrombotic condition had been done for 8 patients, although a positive finding was present only in 4 patients. Their details have been mentioned in table 10.

Bleeding (table 11)

The major sites of bleeding were gastrointestinal (GI), genitourinary (GU), respiratory and cutaneous. One patient each had bleeding from the bone marrow biopsy site and intracranial hemorrhage. Similarly, one patient had presented with left knee hemarthrosis of unknown etiology and was diagnosed to have cellular phase of PMF. GI hemorrhage was the most common site of bleeding and was seen in 14 (6.2%) of patients. This was

most frequent in the cellular phase of PMF 3 (16.7%) and ET 4 (12.9) patients. Genitourinary hemorrhage was seen in one patient of polycythemia and in one patient of probable MPN. Similarly, occasional patients of PV and ET had epistaxis and one patient of PMF had hemoptysis whose anatomic cause could not be determined. Cutaneous bleeding in the form of ecchymosis was also present in isolated patients of ET and PMF. These sites and frequencies of bleeding are similar to previously published data.[104]

Leukemic transformation (table 12)

In the current analysis 5 (2.2%) patients underwent leukemic transformation. This occurred in 1 (3.2%) of patient with ET and 4 (8.3%) patients with PMF. The patient of ET developed acute myeloid leukemia 367 days after being diagnosed with ET. Amongst the 4 patients with PMF who had undergone leukemic transformation the median time from diagnosis of PMF to leukemic transformation was 52 weeks (range 33-365). None of the patients underwent definitive treatment for leukemia and was managed with palliative intent and all subsequently expired. The low rate of leukemic transformation is similar to what has been published earlier.[105]

Progression to myelofibrosis, table 13

A total of 7 (7.3%) patients of PV had progression to myelofibrosis and the time for documentation of progression from the time of diagnosis of PV was known in 5 patients. This ranged from 24-1383 weeks with a median of 537 weeks. Two of these patients had been diagnosed outside to have PV and when they came for review to us their disease had

progressed to myelofibrosis, hence these patients were labeled as post-PV MF. This prevalence too, is similar to what has been published previously.[104]

JAK2V617F mutation positivity (table 14)

Overall JAK2 mutation was positive in 134 (59%) patients. The highest rate of positivity was in PV, 64 (66.7%) which is, however, significantly lower than what has been reported previously. Whether this is due to an increased prevalence of other mutations of the JAK2 gene, or of the thrombopoietin receptor (Mpl) gene needs further investigation. All the patients of post-PV and post-ET MF were JAK2V617F positive.

Cumulative incidence of thrombosis

The cumulative incidence of thrombotic events in PV, ET, PMF and secondary erythrocytosis is depicted in figure 2 and was significantly different between these diseases ($p<0.005$). A significant proportion of patients with ET and PV had developed thrombosis before being diagnosed as having an MPN.

Treatment

Only about 2/3rd of patients with PV and secondary polycythemia underwent venesection as first line treatment. About 1/3rd of patient with PMF received thalidomide with prednisolone as first line therapy. Therapy led to a partial remission in at least 2/3rd of the patients similar to what has been published earlier.[106]

CONCLUSIONS

CONCLUSIONS

- The myeloproliferative disorders are a heterogeneous group of diseases.
- The mean age of PV, ET and PMF was about 10 years less in our analysis.
- There was a significant male preponderance in the PV and PMF group which may be due to the social factors which influence medical care of the women in India.[90, 91]
- Several patients of PV had been diagnosed incidentally while being evaluated for an unrelated reason.
- Except pruritus, which was low in our series, the other clinical features and response to treatment were similar to what has been published in the international literature.
- The incidence of JAK2V617F mutation was lower than what has been reported in the international literature.

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APPENDIX

APPENDIX

Criteria for diagnosis of the Philadelphia negative myeloproliferative disorders as mentioned in the 'WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues', Fourth Edition.

Criteria for the diagnosis of PV

Major criteria
1. Hemoglobin > 18.5 g/dL in men, 16.5 g/dL in women or other evidence of increased red cell volume*
2. Presence of *JAK2*617V>F or other functionally similar mutation such as *JAK2* exon 12 mutation
Minor criteria
1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation in vitro

Diagnosis requires the presence of both major criteria and 1 minor criterion or the presence of the first major criterion together with 2 minor criteria.

* Hemoglobin or hematocrit greater than 99th percentile of method-specific reference range for age, sex, altitude of residence or hemoglobin greater than 17 g/dL in men, 15 g/dL in women if associated with a documented and sustained increase of at least 2 g/dL from an individual's baseline value that can not be attributed to correction of iron deficiency, or elevated red cell mass greater than 25% above mean normal predicted value.

Criteria for the diagnosis of ET

1. Sustained platelet count ≥ 450 × 10^9/L*
2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes; no significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis
3. Not meeting WHO criteria for PV,† PMF,‡ CML,§ MDS,¶ or other myeloid neoplasm
4. Demonstration of *JAK2*617V>F or other clonal marker, or in the absence of a clonal marker, no evidence for reactive thrombocytosis‖

Diagnosis requires meeting all 4 criteria.

* During the work-up period.

† Requires the failure of iron replacement therapy to increase hemoglobin level to the PV range in the presence of decreased serum ferritin. Exclusion of PV is based on hemoglobin and hematocrit levels, and red cell mass measurement is not required.

‡ Requires the absence of relevant reticulin fibrosis, collagen fibrosis, peripheral blood leukoerythroblastosis, or markedly hypercellular marrow for age accompanied by megakaryocyte morphology that is typical for PMF− small to large with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous or irregularly folded nuclei and dense clustering.

§ Requires the absence of *BCR-ABL*.

¶ Requires absence of dyserythropoiesis and dysgranulopoiesis.

‖ Causes of reactive thrombocytosis include iron deficiency, splenectomy, surgery, infection, inflammation, connective tissue disease, metastatic cancer, and lymphoproliferative disorders. However, the presence of a condition associated with reactive thrombocytosis does not exclude the possibility of ET if the first three criteria are met.

Criteria for the diagnosis of IMF

Major criteria
1. Presence of megakaryocyte proliferation and atypia,* usually accompanied by either reticulin and/or collagen fibrosis, or, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (ie, prefibrotic cellular-phase disease)
2. Not meeting WHO criteria for PV,† CML,‡ MDS,§ or other myeloid neoplasm
3. Demonstration of *JAK2*617V>F or other clonal marker (eg, *MPL*515W>L/K), or in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic diseases¶
Minor criteria
1. Leukoerythroblastosis‖
2. Increase in serum lactate dehydrogenase level‖
3. Anemia‖
4. Palpable splenomegaly‖

Diagnosis requires meeting all 3 major criteria and 2 minor criteria.

* Small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei and dense clustering.

† Requires the failure of iron replacement therapy to increase hemoglobin level to the polycythemia vera range in the presence of decreased serum ferritin. Exclusion of polycythemia vera is based on hemoglobin and hematocrit levels. Red cell mass measurement is not required.

‡ Requires the absence of *BCR-ABL*.

§ Requires the absence of dyserythropoiesis and dysgranulopoiesis.

¶ Secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies. It should be noted that patients with conditions associated with reactive myelofibrosis are not immune to primary myelofibrosis and the diagnosis should be considered in such cases if other criteria are met,

‖ Degree of abnormality could be borderline or marked.